Profile of John L. R. Rubenstein

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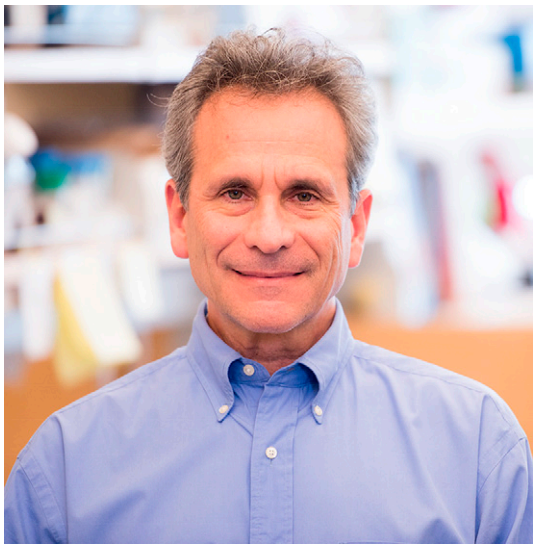
For over three decades, developmental neurobiologist John L. R. Rubenstein has been a leader in research on the forebrain, the seat of higher-order brain functions. The Nina Ireland Distinguished Professor in Child Psychiatry at the University of California, San Francisco (UCSF), Rubenstein has identified transcription factor (TF) genes, transcriptional and signaling pathways, and more recently, TF networks that control regional and cell-type specification in the developing cerebral cortex and basal ganglia. Elected to the National Academy of Sciences in 2020, Rubenstein reports in his Inaugural Article the identification of cortical ventricular zone (VZ) enhancers— short sections of noncoding DNA that regulate gene expression—and a cortical regionalization TF network that regulates cortical regional patterning in radial glial stem cells (1). The findings may provide insights into human neurodevelopmental disorders.



John L. R. Rubenstein. Image credit: Barbara Ries, University of California, San Francisco.

Mentored by Prominent Scientists

Born and raised in California, Rubenstein was influenced by his parents, particularly his father Edward, who was a professor of medicine at Stanford University. "He fostered my interest in science in many ways, including through his own research in hematology and cardiology, and his basic science educational programs that were taught by the likes of Linus Pauling, Arthur Kornberg, and Joshua Lederberg, individuals whom I came to know," says Rubenstein. His mother Nancy was also very supportive of Rubenstein and his siblings, including a sister who was born with a malformation of the central nervous system. His sister's condition later contributed to his clinical interest in trying to improve the lives of children.

While a student at The Thacher School between 1969 and 1973, and later as an undergraduate at Stanford, Rubenstein obtained summer research positions with Stanford cardiac surgeons Norman Shumway and Randall Morris, studying immune responses to heart transplants. As an undergraduate at Stanford, he worked with biochemists Kornberg and William Wickner, studying DNA replication. He also worked on bacteriophage replication with geneticist A. T. Ganesan and on mitochondrial DNA superhelicity with developmental biologist David Clayton and biochemist Douglas Brutlag (2).

Antisense RNA and Gene Expression

During his sophomore year at Stanford, Rubenstein went to a scientific meeting where he heard a lecture on the transcriptomic complexity of brain RNA by University of Michigan geneticist Gilbert Omenn. He says, "This and other factors convinced me to spend my career studying gene expression in the developing brain." Following the advice of neuroscientist John Nicholls, he postponed those investigations until he received more basic science training.

Rubenstein also resolved to become fully trained in medicine. Upon earning a bachelor's degree in chemistry, he entered Stanford's MD/PhD program. His doctoral work in biophysics under Harden McConnell included research on the dynamics of membrane lipids and proteins as a function of cholesterol concentration (3). He also conducted cell biological and molecular studies of membrane biogenesis under James Rothman that involved generating synthetic messenger RNA. Rubenstein says,

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"During this period, I greatly benefited from genetic engineering training that I had obtained from (biochemists) Hiroto Okayama and Paul Berg."

After earning MD/PhD degrees in 1983, Rubenstein became a postdoctoral fellow at the Pasteur Institute in Paris, where he stayed until 1986. Under the guidance of molecular biologists François Jacob and Jean Francois Nicolas, he was among the first to show that antisense RNA can inhibit gene expression (4). With Nicolas and colleague Joshua Sanes of Washington University, he also was among the first to demonstrate that recombinant retroviral vectors can be used to generate transgenic mice (5) and to use such vectors to study cell lineage in developing mic (6).

Studies on Forebrain Organization and Gene Expression

Rubenstein returned to Stanford for an internship in medicine, neurology, and psychiatry in 1986 and residencies in adult and child psychiatry. Psychiatrist and mentor Roland Ciaranello supported him in initiating analysis of forebrain development. Rubenstein devised a method to perform subtraction hybridization to purify TES transcripts that are preferentially expressed in the embryonic compared to the adult telencephalon (7). The method allowed Rubenstein, with trainees Matthew Porteus and Alex Bulfone, to identify *TES-1* (*Dlx2*) and *Tbr1*, respectively. These were among the first known genes with forebrain-specific central nervous system expression.

In 1991, Rubenstein joined the faculty at the UCSF department of psychiatry, where he now serves as a professor. He is exceedingly grateful to the over 100 trainees and collaborators who have made his team's work possible. One such collaboration, with University of Murcia, Spain, neuroanatomist Luis Puelles, led to the prosomeric model in 1994 (8). Based on the expression of several genes, such as *Dlx2* and *Tbr1*, the model described the topological organization of embryonic forebrain divisions. Rubenstein's team subsequently provided evidence that the genes *Fgf8* and *Shh* control regional patterning of the embryonic forebrain (9).

Interneuron Migration

Rubenstein's team discovered and characterized a *Dlx*-dependent core transcriptional program that controls development and function of cortical GABAergic (inhibitory) neurons (10). This work, and a subsequent study (11), demonstrated a revolutionary idea: Cortical interneurons are generated in the basal ganglia and tangentially migrate to the cortex. Rubenstein says, "This discovery has broad implications on cortical developmental biology, genetics, evolution, and disorders. Furthermore, it has led to the idea that interneuron transplantation can be a therapeutic approach."

Systematic genetic and genomic analyses enabled Rubenstein's and University of California, Davis neurogenomicist Alex Nord's teams to define transcriptional pathways controlling differentiation of GABAergic as well as glutamatergic progenitors and neurons, including identification of such a pathway controlled by TF *NKX2-1* (12). The integrated approach provided a foundation for elucidating transcriptional networks guiding development of the medial ganglionic eminence (MGE), a telencephalic progenitor domain, and its descendants.

Identification of Enhancer Elements

The transcriptional pathways controlling differentiation of GABAergic and glutamatergic progenitors and neurons were found to include the gene regulatory elements, termed enhancers, that Rubenstein and his team have extensively studied. In collaboration with University of Ottawa biologist Marc Ekker and Lawrence Berkeley National Laboratory senior scientists Axel Visel and Len Pennachio, he has, since the 1990s, identified over 300 enhancers active in the developing cortex and basal ganglia. Rubenstein says, "This work converges with our ongoing whole-genome analyses of transcription function." For example, they investigated the general mechanisms, including enhancers, by which TFs orchestrate expression programs involved in neurodevelopment (13).

Rubenstein's group showed that enhancer activity can be restricted to specific embryonic domains and cell types (14). He and his team used the findings, in part, to create a high-resolution enhancer atlas of the developing telencephalon. Rubenstein and his team additionally defined a progenitor map of cortical regions (15), which was first postulated in 1988 by Yale neuroscientist Pasko Rakic.

More recently, Rubenstein's and biotechnologist Katherine Pollard's Gladstone Institute/UCSF teams used epigenomics and machine learning to identify brain-specific enhancers. The resulting dataset of around 19,000 regulatory regions of the genome expected to play a role in brain development was incorporated into a new genomic atlas of the developing human brain (16). It is a tool now used by Rubenstein and others to probe the underlying biology of neurodevelopmental disorders.

Mechanisms of Cortical Patterning

A once-widely accepted theory, known as the Tabula Rasa hypothesis, held that cortical regional patterning was strongly influenced by neural activity in the brain's thalamus. A study by Rubenstein and colleagues contradicted that idea, demonstrating that the initial establishment of such patterning is largely independent of thalamic input (17).

A significant aspect of Rubenstein's work on cortical patterning concerns investigating developmental mechanisms underlying autism and epilepsy. A breakthrough occurred in 2001, when he and his team showed that *Tbr1* prenatally specifies identity of the deepest cortical layer (6, 18). This helped explain why a mutation of the gene could result in autismi. Along with colleagues Bin Chen, Matt State, and others, he went on to demonstrate that *Tbr1* postnatally promotes synaptogenesis through WNT signaling (19), such that the *Tbr1* mutant synapse

phenotype can be rescued by promoting WNT signaling with lithium chloride, a finding with potential clinical implications (20).

Father–Son Honors

For his work on cortical development and other achievements, Rubenstein has received numerous awards and honors. For example, he is an elected member of the National American Academy of Arts and Sciences (2016), and he received the Ruane Prize for Outstanding Child and Adolescent Psychiatric Research from the Brain & Behavior Research Foundation (2016). He is especially proud of being an elected member of the National Academy of Medicine (NAM) (2006), since his father also received this honor. Rubenstein says, "We are among the few father–son pairs to have been elected to the NAM."

Defining Transcriptional Circuits

Rubenstein's Inaugural Article (1) represents a shift in his team's research from the functions of single genes toward understanding how transcriptional networks orchestrate forebrain development. Rubenstein says, "An overarching goal is to define transcriptional circuits that control regional and cell-type specification in the cerebral cortex and basal ganglia and to explore their relevance to human neurodevelopmental disorders."

Currently, his team is performing whole-genome analyses to define the regulatory elements and genes controlled by a set of TFs known to have cardinal developmental roles. The research continues to define candidate enhancers whose activity the researchers then characterize using transgenic assays. The work holds promise for deriving components of additional transcription circuits.

Translational Work

Rubenstein's present work also includes serving as a scientific advisor for Neurona Therapeutics, a California-based preclinical-stage biotechnology company that he cofounded in 2008 with fellow UCSF faculty members Arturo Alvarez-Buylla, Arnold Kriegstein, and Cory Nicholas. "Neurona is dedicated to generating human MGE-derived interneurons to treat human epilepsy," he says.

Neurona, as expressed in its mission statement, also plans to create cell therapies for other chronic disorders of the nervous system. In addition to work related to cell therapies for epilepsy (21), Rubenstein has helped show that cell transplantation in rodents can reduce memory loss in an Alzheimer's disease model (22) and neuropathic pain (23).

It will require years of both basic and translational research to reach the many clinical goals, but Rubenstein and his team are up to the challenge. "Like long-distance running, it is not a short-term process to be successful in science. It is a career where you are in it for the long term," he says (24).

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